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Relationship Between Autonomic and Behavioral Thermoregulation in the Mouse

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Oxygen consumption Preferred ambient temperature Evaporative water loss Thermal conductance
Colonic temperature Temperature gradient Ambient temperature

HOMEOTHERMIC animals maintain a stable core temperature through the utilization of behavioral and autonomic thermoregulatory effectors. When given the opportunity to select their environmental temperature, adult mice will select a ambient temperature (T_a) of approximately 31°C [4, 5, 10]. Other studies on the autonomic thermoregulatory mechanism of mice indicate that a T_a of 31°C is very near the thermoneutral zone [8]. That is, the zone of T_a's where metabolic rate and evaporative heat loss are at minimal levels. Increasing T_a above the thermoneutral zone is associated with an increase in evaporation whereas decreasing T_a below the thermoneutral zone is associated with an elevation in metabolic rate. Both of these autonomic responses are essential for maintaining a constant body temperature against changes in T_a.

The relationships between the activity of autonomic thermoregulatory effectors and the preferred T_a in a rodent species has involved comparisons across different studies. No single study has compared the activity of metabolism, evaporation, and thermal conductance to a rodent's preferred T_a. Such measurements are essential in assessing the interaction between the behavioral and autonomic thermoregulatory systems. In this study metabolic rate, evaporative water loss, thermal conductance, and colonic temperature were measured as a function of T_a in mice. The preferred T_a was measured in the same animals using a temperature gradient. The purpose of this study is to measure the preferred T_a of the mouse and to relate these data to the effect of T_a on the level of activity of autonomic thermoregulatory effectors and colonic temperature.

METHOD

Animals

Animals used in this study were young adult, male mice of the BALB/c strain obtained from Jackson Laboratories (Bar Harbor, ME). The mice were approximately 6 weeks of age at the time of testing. The animals were housed in groups of five at a T_a of 22°C, 50% relative humidity and maintained under a 12:12 L:D photoperiod. The animals were provided food (Lab Blox, Wayne Pet Food) and water ad lib. The mean body weight of the mice during the testing period was 24.9 g (range=23.3–27.2 g).

Behavioral Response

Preferred T_a (PT_a) was measured using a temperature gradient similar in design to a previous report [6]. The temperature gradient was constructed from a 96 cm length of square aluminum tubing (70 mm inside diameter). One end of the tube was immersed in a cold water bath maintained at a temperature of 14°C and the other side was immersed in warm water bath maintained at 43°C. A 76 cm length of square aluminum tubing (44 mm inside diameter) which contained the mouse, was placed inside the larger tubing. Holes for light transmission, which served to automatically monitor the position of the animal in the temperature gradient, were placed at 1.6 cm intervals along the length of the tubing. After thermal equilibration inside the large tubing, the small tubing had a high surface temperature of 36°C and a low surface temperature of 18°C. The change in temperature with

distance in the temperature gradient was not perfectly linear. However, a plot of the logarithm of temperature versus distance yielded a linear relationship which was used to convert position in the gradient to PT_a. The position of mice in the temperature gradient was automatically recorded [6] and then converted to PT_a. Dry air was passed through the gradient from the warm-to-cold direction at a flow rate of 1.0 l/min.

Autonomic Responses

In another experimental apparatus oxygen consumption and evaporative water loss (EWL) were continuously recorded from single mice at different T_a's in a stainless steel chamber (inner volume=1.9 l) that was immersed in a temperature-controlled water bath. Air dryed over calcium anhydrite was drawn into the chamber at a rate of 1.1 l/min (STP). A small fraction of the effluent air (120 ml/min) was first drawn through the sensor of a dew point hygrometer (General Eastern, model 1200 APS). A layer of mineral oil placed at the bottom of the environmental chamber prevented moisture from urine and feces from entering into the measurement of the dew point temperature (T_{dp}). After passing through the dew point hygrometer the air stream was dried and then was drawn into an oxygen analyzer (Electrochemistry, model S-3A), which provided a continuous measurement of percent oxygen. T_a inside the chamber was monitored with a YSI temperature probe. Percent oxygen, T_{dp}, and T_a were continuously recorded on a digital-analog strip chart (Dianachart, model DG-5).

Oxygen consumption (ml/min) was calculated by multiplying the rate of air flow by the change in the relative fraction of oxygen before and after passing through the environmental chamber. Metabolic rate in dimensions of W/kg was derived from oxygen consumption by assuming that 1.0 ml of consumed oxygen was equivalent to 20.1 J (i.e., assuming a respiratory quotient of ~0.82).

The rate of whole-body EWL in dimensions of mg/min was calculated from the change in T_{dp} before and after passing through the environmental chamber (e.g., [3]). EWL was then normalized with respect to oxygen consumption in units of mg evaporated water per ml of consumed oxygen. In this way the effect of T_a on the thermoregulatory activation of EWL can be dissociated from the direct effect of metabolic rate on EWL (e.g., [13]).

Through a modification of an equation described by Aschoff [1] and Scholander *et al.* [14], whole-body thermal conductance was calculated according to:

$$C = \frac{MR}{T_b - T_a}$$

where C=thermal conductance, MR=metabolic rate, and T_b=body temperature. Thermal conductance is a measure of the ease of heat transfer from the body core to the environment and is reciprocal of insulation [2].

Protocol

Since naive mice exhibit highly variable responses in the temperature gradient and environmental chamber, it was decided to use the same animals repeatedly with the hope of collecting data with low variabiltiy that would provide a good estimate of the true thermoregulatory response of the species. The nine mice were first placed in the temperature gradient for 90 min daily for four consecutive days. All experiments were performed between 9 a.m. to 5 p.m. During the next two weeks the same mice were placed for 90 min in an environmental chamber maintained at a given T_a. The T_a's ranged from the approximate lower (18°C) and upper limits (34.5°C) of the temperature gradient. Generally, four mice were tested at each T_a. Immediately after the 90 min exposure period in the chamber the colonic temperature was determined by inserting a temperature probe 2.4 cm past the anal sphincter. Colonic temperature was not measured in the behavioral experiments. Each animal was tested randomly during the day in both the behavioral and autonomic experiments.

TABLE 1
PREFERRED AMBIENT TEMPERATURE OF THE BALB/C MICE MEASURED ON FOUR CONSECUTIVE DAYS

	Preferred T_a, °C			
Day	1	2	3	4
Mean	31.9	31.2	29.5	31.1
S.D.	1.7	2.8	1.4	2.4
N	9	9	9	8

Only 8 runs were collected on day 4 due to apparatus malfunction.
S.D.=standard deviation.

Data Analysis

In the behavioral experiments the PT_a measurements (1 measurement/2 min) over the final 30 min of the 90 min testing period were averaged to one value. The mean± S.D. for the nine mice for each day was calculated. In the measurement of autonomic responses, functional relationships between each autonomic parameter (metabolic rate, EWL, and thermal conductance) and T_a were assessed using least-squares regression procedures on a Hewlett-Packard programmable calculator (model 9815A). In addition, the mean± S.D. of colonic temperature and each autonomic parameter were calculated at a given T_a.

It should be noted that in both the behavioral and autonomic experiments, environmental conditions other than temperature were kept as uniform as possible. For example, in both experiments the animals were exposed to dry air at a flow of approximately 1.0 l/min. Both systems were constructed of metal immersed in water baths to achieve good thermal exchange. However, the floor of the metabolic chamber was constructed of a metal grid whereas the floor of the gradient was made of solid aluminum. This difference in construction might alter conductive heat loss in the two chambers at a given T_a. All animals were treated similarly and, in both experiments, animals were tested for 90 min and only the last 30 min of data were averaged and used for analysis.

RESULTS AND DISCUSSION

When first placed in the temperature gradient the mice moved from the warm to cool end repeatedly for at least 30 min as reported previously [6]. Generally, after one hour in the temperature gradient the mice would settle into one location with occasional bouts of movement. The average PT_a of the mice over four sequential days varied from 29.5 to 31.9°C (Table 1). Over the four days the mean PT_a was 30.9°C, which is in good agreement with other studies that

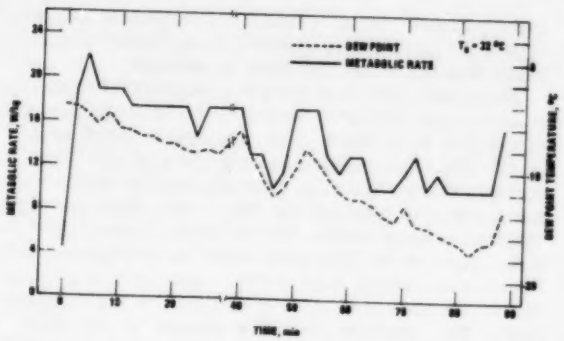

FIG. 1. Time course of metabolic rate (oxygen consumption) and dew point temperature (T_{dp}) of the effluent air of the environmental chamber at a ambient temperature (T_a) of 32°C. Note the transient elevation in metabolic rate and T_{dp} when the animal is placed in the chamber at time 0.

have used a temperature gradient to measure the PT_a of adult mice [4, 5, 10].

The time course of metabolic rate (solid curve) and T_{dp} (dotted curve) at a T_a of 32°C is shown in Fig. 1. This figure illustrates the high initial rates of metabolism and evaporation of mice when they are first placed in the environmental chamber. Generally, after one hour the rates of metabolism and evaporation approached baseline conditions and remained relatively stable. It was thought that the one hour adjustment period would allow the mice to approach thermal equilibrium. This thermal equilibrium may be verified with repeated measurements of core temperature, however, this technique causes stress which further disrupts thermal equilibrium. Thus, it had to be assummed that a stable metabolic rate after one hour in the environmental chamber was conducive of thermal equilibrium.

The overall relationship of colonic temperature, metabolic rate, evaporation and thermal conductance versus T_a is summarized in Fig. 2. Colonic temperature was regulated at a stable level of 36.9 to 37.3°C during exposure to T_a's of 17.8 to 31.1°C. At T_a's of 32 and 34.5°C, colonic temperature increased to 37.5 and 38.0°C, respectively. It would appear that under these laboratory conditions, autonomic responses in the BALB/c mice cannot maintain a normal colonic temperature above T_a's of 32°C. Metabolic rate displayed a typical discontinuous response as a function of T_a within and below the zone of thermoneutrality (e.g., see [14]). Metabolic rate was stable with minimal change between T_a's of 31 to 34.5°C. As T_a decreased below 31°C metabolic rate underwent an approximate linear increase ($MR = -.96\ T_a + 45.5$, $r = 0.89$). The intersection of the regression line with the minimal resting metabolic rate occurred at a T_a 31.8°C, which is defined as the lower critical T_a (e.g., [9]). This value of the lower critical T_a for the mouse is similar to past studies (for review, see [8]). Whole-body evaporation, normalized with respect to oxygen consumption, exhibited a flat response to T_a's of 18 to 29°C but increased markedly above 31°C. A parabolic regression equation was found to provide the best fitting relationship for EWL as a function of T_a ($EWL = 5.5 + -0.36\ T_a + 0.008\ T_a^2$, $r = 0.61$). Other studies have demonstrated that a T_a of 30°C is the approximate critical level where EWL sharply increases in mice [8,15].

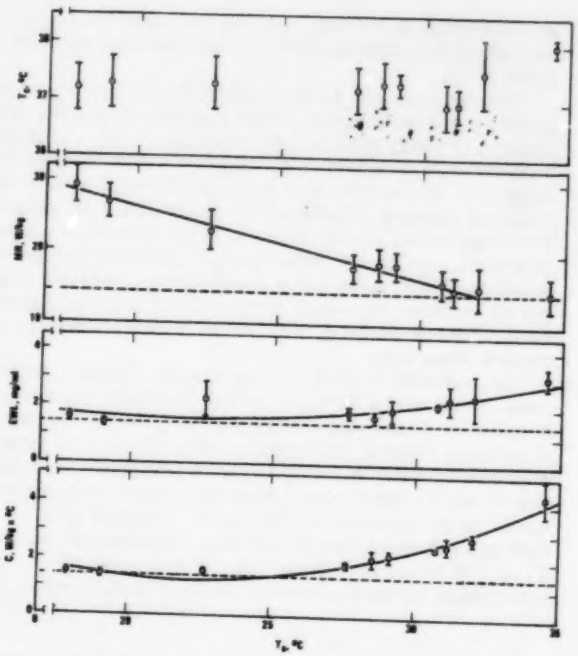

FIG. 2. Overall effect of T_a on colonic temperature (T_c), metabolic rate (MR), evaporative water loss (EWL), and thermal conductance (C) in the BALB/c mouse. Data are plotted as mean±standard deviation. Linear and parabolic lines were fit by least-squares regression (see Results). Dashed lines indicate minimal levels for each parameter.

The ability of rodents to dissipate heat by evaporation is inversely related to the ambient vapor pressure [15]. Thus, in the present study, the use of dryed air in the metabolic measurements probably yields the highest measurement of EWL for the BALB/c mouse. It should be noted that if EWL was not normalized to the rate of oxygen consumption then the EWL vs. T_a response would look similar to the metabolic rate vs. T_a response. By normalizing EWL to oxygen consumption it is possible to dissociate the thermoregulatory activation of EWL from the direct effect of metabolic rate on EWL.

Thermal conductance followed a similar pattern as EWL with increasing T_a. At T_a's of 18 to 22.7°C thermal conductance was a stable 1.5 W/(kg·°C). At T_a's of 27.6°C and above, thermal conductance underwent a steady increase. A parabolic regression equation was found to be the best fitting relationship for thermal conductance as a function of T_a (thermal conductance $= 9.9 - 0.78\ T_a + 0.01\ T_a^2$; $r = .93$). Similar relationships between thermal conductance and T_a have been reported for an array of homeotherms [8].

By comparing the preferred T_a data in Table 1 with the autonomic responses and colonic temperature data in Fig. 2 it is possible to estimate the preferred level of activity of the autonomic effectors and preferred colonic temperature as controlled through the behavior of the mice. Thus, a preferred T_a of 30.0°C is (1) slightly less than the lower critical T_a where metabolic rate increases, (2) associated with an EWL that is 57% higher than the minimal baseline level, (3) associated with a thermal conductance that is 92% higher

than the minimal baseline, and (4) very near the upper T_a limit where deep body (colonic) temperature is maintained at stable levels.

That the preferred T_a for the BALB/c mouse is approximately equal to the lower critical T_a indicates that the mice prefer a thermal environment associated with a minimal metabolic rate. The preferred T_a is several degrees above the range of T_a where EWL is at its stable, minimal level. If the mice had selected a preferred T_a associated with minimal EWL they would have been several degrees below the lower critical T_a, and hence, would have to increase energy expenditure. Thus, under these experimental conditions (e.g., dry air, solitary exposure), mice prefer a thermal environment where a minimal metabolism is preferred over increased water loss.

The preferred T_a of 30.9°C was associated with a thermal conductance of 3.0 W/(kg·°C) which is double the minimal baseline level observed at the lower T_a's. Under conditions of minimal thermal conductance, skin blood flow is at a minimum to conserve heat loss and skin temperature is near that of the T_a. As T_a rises above the critical level, thermal conductance increases as the animal increases skin blood flow and enhances passive heat loss. For example, below a T_a of 27°C blood flow in the tail of the rat is at a very low level which increases abruptly above 27°C [11]. Assuming that the mouse exhibits a similar tail blood flow response [7], then at the preferred T_a of 30.9°C there should be substantial blood flow to the tail and other appendages.

It has been shown in many homeotherms that during exposure to thermal stress behavioral thermoregulation generally takes precedence over autonomic thermoregulation [4,12]. The reason behind this hierarchy of activation is apparently related to energy requirements for behavioral versus autonomic mechanisms; the former generally requires much less energy compared to the latter. In the present study it has been shown that mice select an environmental temperature associated with minimal metabolic requirements, but an elevated level of evaporation and thermal conductance. This provides additional support to the notion that behavioral thermoregulation is closely related to the animal's metabolic requirements.

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